OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Blood RNA 96 Kit

50 µL Blood

M2837-00	1 x 96 preps
M2837-01	4 x 96 preps

200 µL Blood

M2839-00	1 x 96 preps
M2839-01	4 x 96 preps

December 2013

For research use only.Not intended for diagnostic testing.

Mag-Bind® Blood RNA 96 Kit

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Introduction and Overview

The Mag-Bind® Blood RNA 96 Kit is designed for rapid and reliable isolation of total and viral RNA from mammalian whole blood. The Mag-Bind Bead technology provides high-quality RNA, which is suitable for direct use in most downstream applications, such as amplifications and enzymatic reactions. These protocols can be easily adapted to an automated system and the procedure can be scaled up or down.

If using the Mag-Bind® Blood RNA 96 Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. Samples are lysed in a specially formulated buffer containing detergent and chaotropic salt. After adjusting the buffer conditions, nucleic acids (DNA/RNA) will form a complex with magnetic beads. The beads/nucleic acids complex is separated from lysates using a magnet. Proteins and cellular debris are efficiently washed away by a washing step. Next, DNA is removed with a Mag-Bind® DNase I treatment. RNA is rebound and cleaned from the Mag-Bind® DNase I reaction mixture using a second magnetic bead binding and washing procedure. Pure RNA is eluted in nuclease-free water or low ionic strength buffer. Purified RNA can be directly used in downstream applications without the need for further purification.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

- DNase I has been replaced by Mag-Bind® DNase I. This is a name change only.
- Proteinase K is now supplied in a liquid form eliminating the resuspension step prior to use.
- Proteinase K Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips and plastic ware for the supplied reagents.

- Equilibrate samples and reagents to room temperature before beginning this protocol. All steps should be carried out at room temperature unless otherwise noted. Work quickly, but carefully.

- Prepare all materials required before starting the procedure to minimize RNA degradation.

Quantification and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm with a spectrophotometer. One OD unit measured at 260 nm corresponds to 40 µg/mL RNA. DEPC Water is slightly acidic and can dramatically lower absorbance values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while an A_{260}/A_{280} ratio of 0.6 denotes pure protein. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Phenol has a maximum absorbance at 270 nm and can interfere with spectrophotometric analysis of DNA or RNA. Store RNA samples at -70°C in water. Under these conditions, RNA is stable for more than a year.

Integrity of RNA

It is highly recommended that RNA quality be determined prior to beginning all downstream applications. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis with ethidium bromide staining. The ribosomal RNA bands should appear as sharp, clear bands on the gel. The 28S band should appear to be double that of the 18S RNA band (23S and 16S if using bacteria). If the ribosomal RNA bands in any given lane are not sharp and appear to be smeared towards the smaller sized RNA, it is very likely that the RNA undergone degradation during the isolation, handling, or storage procedure. Although RNA molecules less than 200 bases in length do not efficiently bind to the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Kit Contents

Product	M2837-00	M2837-01
Purifications (50 µL blood)	1 x 96	4 x 96
Mag-Bind® Particles CNR	530 µL	2.2 mL
RBL Buffer	7 mL	30 mL
Proteinase K Solution	600 µL	2.4 mL
RXT Wash Buffer	22 mL	88 mL
RNA Wash Buffer II	25 mL	100 mL
DEPC Water	10 mL	40 mL
Mag-Bind® DNase I	110 µL	440 µL
DNase I Digestion Buffer	6 mL	24 mL
User Manual	✓	✓

Product	M2839-00	M2839-01
Purifications (200 µL blood)	1 x 96	4 x 96
Mag-Bind® Particles CNR	2.1 mL	8.4 mL
RBL Buffer	28 mL	120 mL
Proteinase K Solution	2.4 mL	9 mL
RXT Wash Buffer	55 mL	220 mL
RNA Wash Buffer II	50 mL	140 mL
DEPC Water	15 mL	60 mL
Mag-Bind® DNase I	225 µL	900 µL
DNase I Digestion Buffer	12 mL	48 mL
User Manual	✓	✓

Storage and Stability

All Mag-Bind® Blood RNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as recommended. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store at 2-8°C. Mag-Bind® Particle CNR must be stored at 2-8°C. Mag-Bind® DNase I must be stored at -20°C. Store all other components at room temperature (22-25°C). Check buffers for precipitates before use. Redissolve any precipitates by warming to 37˚C.

Preparing Reagents

1. Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M2837-00	100 mL
M2837-01	400 mL
Kit	**100% Ethanol to be Added**
M2839-00	200 mL
M2839-01	560 mL

2. Dilute RXT Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M2837-00	28 mL
M2837-01	112 mL
Kit	**100% Ethanol to be Added**
M2839-00	70 mL
M2839-01	280 mL

Mag-Bind® Blood RNA 96 Kit Protocol (M2837) - 50 µL blood

Materials and Equipment to be Supplied by User:

- Magnetic separation device for 96-well microplates (Recommend Cat#MSD-01)
- Nuclease-free 500 µL 96-well microplates (Recommend Cat# EZ9604-01)
- Multichannel pipette
- Nuclease-free pipette tips
- 100% ethanol
- Isopropanol
- Sealing film

Before Starting:

- Prepare RNA Wash Buffer II and RXT Wash Buffer according to the "Preparing Reagents" section on Page 6.

1. Add 65 µL RBL Buffer and 65 µL isopropanol to each well of a 500 µL 96-well microplate.

2. Add 50 µL blood sample to each well and shake for 1 minute.

3. Add 5 µL Proteinase K Solution and 5 µL Mag-Bind® Particles CNR to each well. Pipet up and down 10 times and shake for 5 minutes to mix thoroughly.

   Note: Proteinase K Solution must be added after the blood sample has been added to RBL Buffer. Mag-Bind® Particles CNR and Proteinase K Solution can be made as a mastermix.

4. Let sit at room temperature for 10 minutes.

5. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

6. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

7. Remove the plate containing the Mag-Bind® Particles CNR from the magnetic separation device.

8. Add 200 µL RXT Wash Buffer to each well. Pipet up and down 20 times or shake for 2 minutes to mix thoroughly.

   Note: RXT Buffer must be diluted with ethanol before use. Please see Page 6 for instructions.

9. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

10. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

11. Remove the plate containing the Mag-Bind® Particles CNR from the magnetic separation device.

12. Repeat Steps 8-11 for a second RXT Wash Buffer wash step.

13. Add 200 µL RNA Wash Buffer II to each well. Pipet up and down 10 times or shake for 2 minutes to mix thoroughly.

    Note: RNA Wash Buffer II must be diluted with ethanol before use. Please see Page 6 for instructions.

14. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

16. Leave the tube on the magnetic separation device for 3 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

17. Prepare the Mag-Bind® DNase I digestion mix as detailed in the table below:

    Note: If total nucleic acid (DNA and RNA) is desired, skip Mag-Bind® DNase I digestion steps (Steps 18-24) and proceed to Step 25 for isolating both DNA and RNA.

Number of Samples	Mag-Bind® DNase I Digestion Buffer	Mag-Bind® DNase I	Total Volume
1	49 µL	1.0 µL	50 µL
4	215 µL*	4.4 µL*	219.4 µL
10	539 µL*	11 µL*	550 µL
96	5.18 mL*	106 µL*	5.29 mL

** Volumes are calculated 10% extra to offset pipetting error.*

Important Notes:

- Mag-Bind® DNase I is very sensitive and prone to physical denaturation. **Do not vortex the Mag-Bind® DNase I mixture.** Mix gently by shaking the plate.
- Freshly prepare Mag-Bind® DNase I digestion mix right before RNA isolation.
- All steps must be carried out at room temperature. Work quickly, but carefully.

18. Add 50 µL Mag-Bind® DNase I digestion mix. Pipet up and down 20 times or shake gently for 2 minutes to mix.

    Note: It is very important to remove any liquid drop from the wells before adding the Mag-Bind® DNase I digestion mix. Mag-Bind® DNase I digestion mix must be used immediately once it is prepared.

19. Let sit at room temperature for 10-15 minutes.

20. Add 200 µL RNA Wash Buffer II. Pipet up and down 20 times or shake for 5 minutes to mix.

21. Let sit at room temperature for 5 minutes.

22. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

23. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

24. Remove the plate containing the Mag-Bind® Particles CNR from the magnetic separation device.

25. Add 300 µL RNA Wash Buffer II to each well. Pipet up and down 10 times or shake for 2 minutes to mix thoroughly.

26. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

27. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

28. Leave the tube on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

29. Add 20-50 µL DEPC Water. Pipet up and down 20 times or shake for 1 minute to mix thoroughly.

30. Let sit at room temperature for 3 minutes.

31. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

32. Transfer the cleared supernatant containing purified RNA into a new RNase-free microplate.

33. Store eluted RNA at -70°C.

Note: Any combination of the following steps can be used to help increase RNA yield.

- Heat the DEPC Water to 70°C before adding to the beads.
- Increase the incubation time to 5 minutes.
- Increase the elution volume.
- Repeat the elution step with fresh DEPC Water (this may increase the yield, but decrease the concentration).

Mag-Bind® Blood RNA 96 Kit Protocol (M2839) - 200 µL blood

Materials and Equipment to be Supplied by User:

- Magnetic separation device for 96-well microplate (Recommend Cat#MSD-01)
- Nuclease-free 1 mL or 2 mL 96-well deep-well plate (Recommend Cat# EZ9602-01)
- Nuclease-free 96-well microplate (Recommend Cat# EZ9604-01)
- Multichannel pipette
- Nuclease-free pipette tips
- 100% ethanol
- Isopropanol
- Sealing film

Before Starting:

- Prepare RNA Wash Buffer II and RXT Wash Buffer according to the "Preparing Reagents" section on Page 6.

1. Add 260 µL RBL Buffer and 260 µL isopropanol to each well of a 500 µL 96-well microplate.

2. Add 200 µL blood sample to each well and shake for 1 minute.

3. Add 20 µL Proteinase K Solution and 20 µL Mag-Bind® Particles CNR to each well. Pipet up and down 10 times and shake for 5 minutes to mix thoroughly.

   Note: Proteinase K Solution must be added after the blood sample has been added to RBL Buffer. Mag-Bind® Particles CNR and Proteinase K Solution can be made as a mastermix.

4. Let sit at room temperature for 10 minutes.

5. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

6. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

7. Remove the plate containing the Mag-Bind® Particles CNR from the magnetic separation device.

8. Add 600 µL RXT Wash Buffer to each well. Pipet up and down 20 times or shake for 2 minutes to mix thoroughly.

   Note: RXT Buffer must be diluted with ethanol before use. Please see Page 6 for instructions.

9. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

10. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

11. Remove the plate containing the Mag-Bind® Particles CNR from the magnetic separation device.

12. Repeat Steps 8-11 for a second RXT Wash Buffer wash step.

13. Add 600 µL RNA Wash Buffer II to each well. Pipet up and down 10 times or shake for 2 minutes to mix thoroughly.

    Note: RNA Wash Buffer II must be diluted with ethanol before use. Please see Page 6 for instructions.

14. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

16. Leave the tube on the magnetic separation device for 3 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

17. Prepare the Mag-Bind® DNase I digestion mix as detailed in the table below:

    Note: If total nucleic acid (DNA and RNA) is desired, skip Mag-Bind® DNase I digestion steps (Steps 18-24) and proceed to Step 25 for isolating both DNA and RNA.

Number of Samples	Mag-Bind® DNase I Digestion Buffer	Mag-Bind® DNase I	Total Volume
1	98 µL	2 µL	100 µL
4	431 µL*	8.8 µL*	439.8 µL
10	1078 µL*	22 µL*	1100 µL
96	10.35 mL*	211 µL*	10.56 mL

** Volumes are calculated 10% extra to offset pipetting error.*

Important Notes:

- Mag-Bind® DNase I is very sensitive and prone to physical denaturation. **Do not vortex the Mag-Bind® DNase I mixture.** Mix gently by shaking the plate.
- Freshly prepare Mag-Bind® DNase I digestion mix right before RNA isolation.
- All steps must be carried out at room temperature. Work quickly, but carefully.

18. Add 100 µL Mag-Bind® DNase I digestion mix. Pipet up and down 20 times or shake gently for 2 minutes to mix.

    Note: It is very important to remove any liquid drop from the wells before adding the Mag-Bind® DNase I digestion mix. Mag-Bind® DNase I digestion mix must be used immediately once it is prepared.

19. Let sit at room temperature for 10-15 minutes.

20. Add 400 µL RNA Wash Buffer II. Pipet up and down 20 times or shake for 5 minutes to mix.

21. Let sit at room temperature for 5 minutes.

22. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

23. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

24. Remove the plate containing the Mag-Bind® Particles CNR from the magnetic separation device.

25. Add 450 µL RNA Wash Buffer II to each well. Pipet up and down 20 times or shake for 2 minutes to mix thoroughly.

26. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

27. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

28. Leave the tube on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

29. Add 100 µL DEPC Water. Pipet up and down 20 times or shake for 1 minute to mix thoroughly.

30. Let sit at room temperature for 3 minutes.

31. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

32. Transfer the cleared supernatant containing purified RNA into a new RNase-free microplate.

33. Store eluted RNA at -70°C.

Note: Any combination of the following steps can be used to help increase RNA yield.

- Heat the DEPC Water to 70°C before adding to the beads.
- Increase the incubation time to 5 minutes.
- Increase the elution volume.
- Repeat the elution step with fresh DEPC Water (this may increase the yield, but decrease the concentration).

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Low RNA Yield	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use
	RNA degraded during sample storage	Make sure samples are stored properly and that the samples are processed immediately after collection or removal from storage
	RNA Wash Buffer II was not prepared correctly	Prepare RNA Wash Buffer II by adding ethanol according to the instructions
	Loss of magnetic beads during procedure	Increase the bead collection time
	Blood clots cause congregation of magnetic beads	Make sure the sample is clear of blood clots before adding magnetic beads.
Problem	Cause	Solution
No RNA eluted	RNA Wash Buffer II was not diluted with 100% ethanol	Prepare RNA Wash Buffer II by adding ethanol according to the instructions
Problem	Cause	Solution
Problem with downstream applications	Insufficient RNA was used	RNA is already degraded; always use fresh blood for RNA isolation
		Quantify the purified RNA accurately and use sufficient RNA
Problem	Cause	Solution
Carryover of magnetic beads during elution	Carryover of magnetic beads in the eluted RNA will not effect downstream applications	To remove the carryover magnetic beads from eluted RNA, simply magnetize the magnetic beads and carefully transfer to a new plate
Problem	Cause	Solution
DNA contamination	Inefficient DNase I digestion	Make sure to use proper starting material
		Ensure that the DNase I digestion is carried out at room temperature

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
E-Z 96 Magnetic Stand	MSD-01
E-Z 96 Magnetic Stand (Radial)	MSD-01b
Magnetic Separation Device	MSD-02
96-well Square-well Plate (2.2 mL), 5/pk	EZ9602-01
96-well Round-well Plate (1.2 mL), 10/pk	SSI-1780-00
96-well Microplate (500 µL)	EZ9604-01
DEPC Water (30 mL)	PR032
RNA Wash Buffer II (20 mL)	PDR046

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